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Abstract

Overweight and obesity are major risk factors for a number of chronic diseases, including diabetes, cardiovascular diseases, and cancer. Obesity rates are on the rise worldwide with women more frequently affected than men. Hedonic responses to food seem to play a key role in obesity, but the exact mechanisms and relationships are still poorly understood. In this study, we investigate the perceived pleasantness of food rewards in relation to satiety and calories consumed during an ad libitum meal in women. Using functional magnetic resonance imaging (fMRI) and a milkshake consumption task, we studied how experienced food values are encoded in women with healthy weight, overweight or obesity. Participants rated the pleasantness and intensity of high and low caloric milkshakes in the fMRI scanner during both the fasted and fed states. We found differences in the neural responses and experienced pleasantness of high and low caloric milkshakes depending on satiety and Body Mass Index (BMI). Women with both high ad libitum consumption levels and high BMI reported greater experienced pleasantness for milkshakes. In contrast, among women

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with low ad libitum consumption levels, greater BMI was associated with less experienced pleasantness. At the neural level, satiety affected women with obesity to a lesser degree than women with healthy weight. Thus, having obesity was associated with altered relationships between food consumption and the hedonic responses to food rewards as well as reduced satiety effects in women.
Keywords: `BMI, Food Pleasantness, Reward`

Introduction

According to the World Health Organization, worldwide obesity has nearly tripled between 1975 and 2016. In 2016, about 13% of the worlds adult population was affected by obesity, with more women (15%) affected than men (11%) (WHO, 2018). Furthermore, projections by the Organization for Economic Co-operation and Development (OECD) predict a further increase of obesity rates by 2030 in most member countries (OECD, 2017). The causal mechanisms of obesity are complex and include multiple physiological, psychological, and social factors. However, it has been hypothesized that two of the key factors in overeating are greater hedonic utility for high caloric foods and reduced satiety effects (Swinburn et al., 2009; Hall et al., 2011).

At the neural level, several regions are associated with calories consumed to reach satiety and satiety, including the amygdala, striatum, orbitofrontal cortex (OFC), insula, and the hypothalamus. The insula and hypothalamus are thought to be important for interoceptive and homeostatic aspects of eating, respectively (Craig, 2002; Naqvi et al., 2014; Berthoud et al., 2017). The amygdala, insula, OFC, and striatum are implicated in hedonic eating and drinking (Saper et al., 2002; Plassmann et al., 2008; Berthoud et al., 2017). Hedonic eating is promoted by experiencing the smells, tastes, and textures of food and it can override homeostatic satiety signals

in some cases (Saper et al., 2002; Lowe et al., 2009). Several studies have shown changes in the hedonic system in people with obesity when they are exposed to food cues (reviewed in Van Vugt (2009); Leng et al. (2017)). However, the neural mechanisms underpinning satiety and overeating in the context of experienced food values, rather than visual food cues, are only beginning to be understood. Previous positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) studies on liquid meals and food odors have shown stronger activation in reward-associated brain regions in response to both the taste (DelParigi et al., 2005) and the smell of foods (Bragulat et al., 2010) in adults with obesity compared to adults with healthy weight in the fasted state. These findings support the idea that the hedonic system may play an important role in triggering higher calories consumption during periods of hunger.

Some studies of experienced food rewards in obesity have focused on neural responses in fasted participants, e.g. Stice et al. (2008); Ng et al. (2011); Devoto et al. (2018). Other studies compared responses to food cues between fed and fasted states in both individuals with obesity and with healthy weight (Martin et al., 2010; Dimitropoulos et al., 2012; Rijn et al., 2015; Yousuf et al., 2018). However, to date few studies have reported within subjects comparisons of experienced food rewards across fasted and fed states in both participants with healthy weight and with obesity. Yet, these data and comparisons are critical for determining how the influence of satiety on the hedonic system may differ in individuals with obesity.

In this study we measure the behavioral and neural responses of women with and without obesity to food rewards experienced in the fasted and fed states. We focused on women in part because they are at a higher risk for obesity but also because they are less well studied (Kanter & Caballero, 2012; Mauvais-Jarvis, 2015; Leeners et al., 2017). We used functional magnetic resonance imaging together with

a task that to measured various components of the hedonic response to experienced food rewards. Specifically, participants rated the pleasantness of low and high caloric milkshakes during different levels of satiety (fasted or fed) on two separate days. While women with healthy weight showed significant differences across satiety states, women affected by overweight or obesity showed neural responses to food rewards in the fed state that are more similar to their responses in the fasted state. In addition, women with both high ad libitum consumption levels and high BMI reported greater experienced pleasantness for milkshakes regardless of satiety state.

Experimental Procedures

Participants

We recruited a group of 72 women for this study. Three women withdrew from the study before completing it, and another was excluded because her German was not sufficiently fluent to understand the tasks. Two more participants had to be excluded because of technical difficulties with the experimental task. Thus, in total we collected a complete data-set from 66 German-speaking pre-menopausal women (age 18-40; mean: 25.7) with regular menses (cycle length mean: 28.8, SD: 2.5). We analyzed body mass index as a continuous measure, but there were approximately 30 women for both the lean and obese group at the time of enrollment. The sample size was based on previous fMRI studies (Little et al., 2008; Lassman et al., 2010; Silva et al., 2011; Schlogl et al., 2013). The study was approved by the Zurich Cantonal Ethics Committee. All participants gave written informed consent and received 500 CHF for their time.

Questionnaires

Women were selected during a general and gynecological screening phase where their medical history was taken. Women with irregular menses, on birth control or hormonal medication, with life history of eating disorders or being recently on diet were excluded (for a complete list of inclusion and exclusion criteria see Appendix). Moreover, during this phase the participants completed three questionnaires, the International Physical Activity Questionnaire (IPAQ) (NIH, 1996; Rütten et al., 2003) on physical activity at work, at home, and during leisure time; The Three-Factor-Eating-Questionnaire (TFEQ) which examines three psychological constructs affecting human eating behaviour, namely cognitive restraint, propensity for disinhibition and hunger (Stunkard & Messick, 1985; Pudel & Westenhöfer, 1989; Lowe et al., 2013); and the Eating Disorder Examination-Questionnaire (EDE-Q) which quantifies sub-clinical abnormalities in eating related to increased risk for psychiatric eating disorders (Beglin & Fairburn, 1992; Thiel et al., 1997; Hilbert et al., 2012). The main group differences concerned lower physical activity, higher cognitive restraint, overall less satisfaction and more concern of body shape and weight for the women with obesity or overweight compared with healthy weight. The groups were similar in income and education level.

Furthermore, both before and after eating, participants provided mood ratings and answered on an anchored rating scale eating-related questions two times inside the scanner and two times outside the scanner. These questions were based on Blundell et al. (2010) and consisted of items such as satiety, desire to eat, and hunger level.

Body mass and physiological measures

We measured the weight of our participants at the time of initial screening and on both days of the experiment. Some participants showed an increase or decrease in Body Mass Index (BMI, weight in kg/height in m^2), between the initial screening and experiment participation such that they were classified as overweight (BMI: 25-30) instead of healthy weight or obese at the time of data collection. In total, our sample consisted of 32 women with healthy weight (BMI mean: 21.9), 10 with overweight (BMI mean: 28.2) and 24 with obesity (BMI mean: 33) based on their weight on the first day of the study (see Appendix Fig. A1). Instead of splitting the participants into two or three different groups, the BMI was used as a continuous covariate (although for better illustration, we sometimes plot healthy versus overweight/obese separately in the figures).

Blood samples ($\sim$ 5 mL) were drawn immediately before the meal and at 6, 12, 28, 24 and 30 min thereafter. These blood samples were used to track the plasma levels and dynamics of intestinal hormones involved in the satiety process as well as to check the menstrual cycle hormones.

At the end of the second day participants performed a gustatory-capacity test. This was designed to detect individual differences in gustatory function that affect hedonic judgment and ad libitum consumption level (Bartoshuk et al., 2006; Dinehart et al., 2006; Hayes et al., 2008; Coldwell et al., 2013). Participants had to rate the sensory intensity of three bitter tastes (0.032 mM, 0.32 mM and 3.2 mM 6-n-propylthiouracil [PROP]) and three salty tastes (0.01 M, 0.1 M and 1 M NaCl). To rate the participants used an unstructured scale similar to the one for the pleasantness and intensity ratings.

Task design

The procedure was the same for each session on both days. Participants performed three different tasks in the scanner. In the task described here, they consumed and rated high and low caloric milkshakes. In the two other tasks, the participants indicated their willingness to pay and willingness to exert physical effort for 30 different food items (these results will be reported in a separate paper). The three tasks were completed four times, i.e. in two menstrual cycle phases (preovulatory or postovulatory), and levels of satiety (fasted or fed). Participants arrived at the scanner after an overnight fast. Specifically, they were instructed not to eat or drink anything after 10 pm on the evening before coming to the lab. They performed the tasks a first time and then received an ad libitum meal before entering the scanner again (time between the two sessions around one hour). During this meal, participants had 30 minutes to eat to satiety from a plate full of ham sandwiches served with water. We instructed the participants to eat until comfortably full.

During the milkshake taste task in the scanner, participants received a 0.1 mL sample of either chocolate or strawberry-flavoured milkshake (see Appendix for the ingredients). The experiment consisted of 4 runs, each with 30 trials (samples), 10 trials per question type (pleasantness, intensity, and identification) for a total of 120 trials in 22 minutes. Each trial had the same structure, first the participant saw a visual cue that a sample of liquid would soon be delivered (2.5 s), which coterminated with the (2 s) delivery of the liquid. Next, a blank screen appeared for 0.5 seconds, followed by a question and response screen, which was displayed for 3.5 seconds. Finally, an instruction to swallow appeared for 1.5 seconds, followed by a fixation cross that signaled the end of the trial (see Fig.1).

The question participants answered on each trial concerned either the pleasantness, identity, or intensity of the liquid. Each question was asked on one third of the

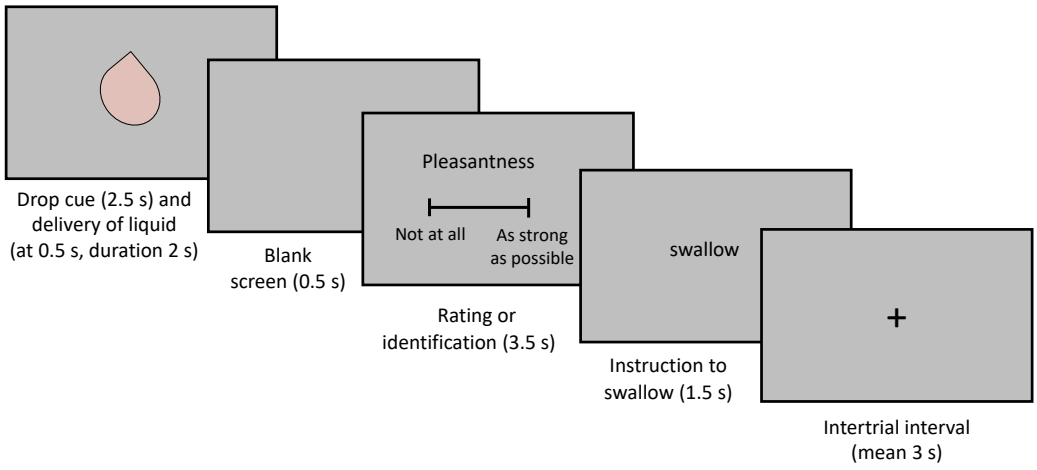

Figure 1: Trial structure, exemplified with a pleasantness question. Participants were asked to rate the pleasantness or intensity of the milkshakes or to identify the flavor (strawberry or chocolate) of the milkshake. Each trial lasted 11 sec.

trials. The pleasantness and intensity of the liquids were rated using a generalized visual analog scale (gVAS (Hayes JE, 2013)). This is a linear scale anchored at the minimum and maximum (see Appendix Fig. A2). These extremes were marked with "not at all" and "as strong as possible" (labels were shown in German), the scale was continuous (from -138 to 138 pixels) and participants made their responses by moving a trackball along the scale and clicking. The responses were measured in pixels, which were normalized to a scale of 0 - 1, extremes included. The "not at all" and "as strong as possible" labels were randomised to the left and right sides of the scale across trials.

When asked about the identity of the liquid, participants had to choose between three options. The options, strawberry, chocolate or water, were displayed on the

screen and the participant had to choose by clicking on one of them using the trackball. In this case the entire scale length (360 pixels) was divided into three sections of 120 pixels, each of them representing one of the three options. The locations (left, middle, right) of the three options were randomized on the unstructured scale for each trial.

Milkshake contents and delivery

The milkshakes were made from a commercial formula mixed with either cream (336 kcal per 100 mL) or water (0 kcal per 100 mL). We mixed two parts of milkshake with one part of either cream or water, to create high and low caloric versions, respectively. In total there were four milkshakes, two high caloric and two low caloric as well as a tasteless artificial saliva solution (KCl 1.86 g/L and NHCO3 0.21 g/L). All liquid amounts were controlled by a custom-built set of pumps and delivered to participants via a mouthpiece during scanning. We used the LabDos Vario Peristaltic Pump from Hitec Zang and fine tuned the setup using special high-quality plastic tubing to get lower rates (5 mL/min) than the minimum specified by the manufacturer (see LabDos (Retrieved 2020)). Each liquid was delivered through one of the 5 different tubes that converged into a mouthpiece. After receiving each liquid, participants had to answer one of three different questions, about the pleasantness, intensity or identity of the experienced milkshake (see below). Therefore, in total each participant received 30 samples per run (3 questions x 5 flavours x 2 repetitions). Each flavour occurs twice per run, so for 4 runs we have 8 samples for each flavour type (including artificial saliva). The order of the milkshake trials was randomized for each participant and the five pumps used to deliver the milkshake were randomized four times during the course of the entire experiment to account for any small mechanical differences among the five pumps.

Primary behavioural data analysis

The behavioral analysis was performed in R v3.5.1 using Rstudio (RStudio Team, 2015; R Core Team, 2018). We focused on the ratings of milkshake pleasantness in the current work in order to test hypotheses related to hedonic experiences. The data followed an extreme values distribution, as participants often selected the extremes (0 or 1) of the rating scale. Inspection of the Quantile-Quantile plot indicated that the assumption of normality did not hold, making linear regressions unsuitable. We therefore used a beta regression model (Cribari-Neto & Zeileis, 2010). This type of regression uses different gamma distributions to model data with extreme values. Moreover, the ratings were converted using the transformation function of Smithson and Verkuilen (Smithson & Verkuilen, 2006). This function shrinks all the data so that there are no 0 or 1 values, e.g. 0.998 instead of 1. The new data y_{Conv} are computed as $y_{Conv} = \frac{(y*(n-1)+0.5)}{n}$, where y are the original data and n is the number of samples.

To account for repeated measurements, we used linear mixed effects models (Laird & Ware, 1982) with which we analyzed pleasantness ratings as a function of the milkshake's caloric content as well as the individual's satiety state and body mass index (see the equation below and 1 for details on the model specification and factors). Specifically, we used a glmmTMB (Generalized Linear Mixed Models using Template Model Builder (Brooks et al., 2017)) model, which is an extension of a beta regression model that allows for random effects terms. For example, the random effect, "Participant" takes into account that multiple ratings belong to the same participant. Thus, the use of a mixed effects model allowed us to fit the data without averaging within participants beforehand. General Linear Mixed Models predict a function of the mean, not the mean itself. As our data were non-Gaussian, we used the beta function with a logit link to simulate the beta regression analysis in

glmmTMB.

The mixed effect model has the form $\mathbf{y} = \mathbf{X}\beta + \mathbf{Zu} + \mathbf{e}$, where y is the dependent variable (pleasantness rating in our case), X is a design matrix for the fixed effects (β) and Z is a block-diagonal design matrix for the random effects (u). The last term e represents the residuals. In particular the primary model with BMI was as follows (in the WHR-model, we replaced BMI with WHR, see below and appendix):

$$\begin{aligned} PleasantnessRating_{ij} = {} & (\beta_0 + u_{0j}) + (\beta_1 + u_{1j})Satiety_{ij} + (\beta_2 + u_{2j})Day_{ij} + \\ & (\beta_3 + u_{3j})Milk_{ij} + \beta_4 TrialNumber_{ij} + \beta_5 AdLibConsumLevel_{ij} + \\ & \beta_6 BMI_{ij} + \beta_7 Satiety_{ij}Milk_{ij} + \beta_8 Satiety_{ij}AdLibConsumLevel_{ij} + \\ & \beta_9 Satiety_{ij}BMI_{ij} + \beta_{10} Satiety_{ij}Day_{ij} + \beta_{11} Milk_{ij}BMI_{ij} + \\ & \beta_{12} Milk_{ij}AdLibConsumLevel_{ij} + \beta_{13} TrialNumber_{ij}BMI_{ij} + \\ & \beta_{14} TrialNumber_{ij}Milk_{ij} + \beta_{15} AdLibConsumLevel_{ij}BMI_{ij} + \\ & \beta_{16} AdLibConsumLevel_{ij}Day_{ij} + \beta_{17} TrialNumber_{ij}Milk_{ij} + \\ & \beta_{18} Milk_{ij}Satiety_{ij}BMI_{ij} + \beta_{19} Milk_{ij}AdLibConsumLevel_{ij}BMI_{ij} + \\ & \beta_{20} Satiety_{ij}AdLibConsumLevel_{ij}BMI_{ij} + \beta_{21} CP_{ij} + \beta_{22} Prop_{ij} + e_{ij} \end{aligned} \tag{1}$$

where i represents the trial, and j indicates the participant. The abbreviation CP stands for cycle phase and Milk for milkshake. Both BMI and Ad Libitum Consumption Level were z-scored at the participant level to make the interpretation of the glmmTMB results easier and to facilitate model convergence. We report detailed information about the factors used in this model in Table 1. In addition to the main effects, we included two and three-way interaction terms to account for potential dependencies between variables in explaining pleasantness ratings. In terms of the random effects, we included random intercepts for subjects, as well

as subject-specific random slopes for all variables. We calculated p-values for the behavioural regression z-scores using the Wald statistic (Wald, 1943).

To test if the effects were robust to potential influences of time on task or practice, we included in the model a regressor and its interactions to account for any trial effects. These regressors quantify if and how evaluations of milkshake pleasantness changed over time. There was a small, but significant decrease in pleasantness ratings over the course of each day. Critically, however, adding the trial effects regressor did not change the main findings.

To visualize the interaction effects of the behavioral model we created illustrative plots with the *ggemmeans* (Ldecke, 2018) and *interact_plot* (Long, 2019) R functions. The visual inspection of the residuals plot did not reveal any obvious deviations from homoscedasticity or normality (Appendix Fig. A3). Furthermore, model diagnostics with the package DHARMa (Hartig, 2019) using simulated residuals confirmed the absence of significant outliers (test based on exact binomial test, $p > 0.999$).

Robustness checks and post-hoc behavioural data analysis

We also conducted a post-hoc analyse on the pleasantness ratings. We replaced BMI with Waist Hip Ratio (WHR) as an index of body composition. The two measures are highly correlated, which prohibited including both in the same regression analysis. However, WHR provides somewhat different information than BMI, particularly with regard to adipose tissue distribution (Leeners et al., 2017). The results of the WHR model were generally consistent with the BMI model, with some additional significant results reported in Table A1.

Magnetic Resonance Imaging data acquisition and preprocessing

Whole-brain fMRI data were acquired using a Philips Achieva 3T whole-body scanner (Philips Medical Systems). We used an 8-channel Philips sensitivity-encoded

Factor name	Type	Levels	Levels label
Milkshake Calories (Milk)	Binary	0 & 1	MilkshakeLow & MilkshakeHigh
Satiety	Binary	0 & 1	Fasted & Fed
BMI	Continuous		
AdLibConsumLevel	Continuous		
Cycle Phase (CP)	Binary	0 & 1	PreOvulatory & PostOvulatory
Prop	Continuous		
Day	Binary	0 & 1	Day1 & Day2
TrialNumber	Continuous		

Table 1: This table provides details on each factor used in the glmmTMB. Here we list the factor name, the type (binary or continuous) as well as the number of levels in the factor and their labels. Our primary regressors of interest were Milkshake Calories, Satiety state, and BMI. We also included the participants' Ad Libitum Consumption Level as another individual characteristic. The Ad Libitum Consumption Level was quantified by weighing the plate with the sandwiches before and after eating and then converting grams to kcal (about 1.8 kcal/g). There were four more control variables, Cycle Phase, Prop, Day and TrialNumber, to account for potential variability across subjects in terms of hormonal status, taste sensitivity (via Prop ratings), potential variability between the first and second days of the experiment and time effect (via TrialNumber, number of drops received). For the Prop ratings, we used the intensity responses to the middle concentration value (0.32 mM) of 6-n-propylthiouracil (aka Prop) because that level provided more variation across participants than the lowest and highest concentrations. Cycle phase assignments were based on cycle monitoring and were coded as preovulatory or postovulatory based on whether the test occurred before or after mid-cycle. The cycle phase was confirmed using hormone levels (estradiol, progesterone and luteinizing hormone) in 60 out of 66 women. In the remaining 6 women the hormones were inconclusive. The effects related to BMI, satiety, and ad libitum consumption level all remain significant when excluding these six women.

(SENSE) head coil. Three-dimensional T1-weighted anatomical scans were acquired with resolution of 1 mm^3 voxels (3D FFE T1 sequence). Due to a scanner update in the middle of the data acquisition, the T2*-weighted images used for BOLD scanning were acquired with slightly different repetition times (TR) before and after the update, 2370 ms and 2381 ms, respectively. In both cases, the echo time (TE) was 30 ms, Flip angle 90°, 40 oblique slices acquired in ascending order with 0.5 mm gap, angulation mid-slice [0°, 0°, -20°] Anterior Posterior, Feet Head, Right Left (AP, FH, RL), 3 × 3 × 3 mm voxel size covering the whole brain, Field Of View [240 mm, 139.50 mm, 240 mm] AP, FH, RL. Every individual participant completed all scanning sessions either before or after the scanner update took place.

The fMRI data were pre-processed with Statistical Parametric Mapping SPM 12 (Henson, 2003; Friston et al., 2007). After discarding the first 5 dummy volumes, images were realigned, unwarped and slice-time corrected (to the middle slice acquisition time). T1-weighted structural images were co-registered with the mean functional image and normalized to the standard T1 MNI template based on the Montreal Neurological Institute (MNI) reference brain, using the segment procedure provided by SPM 12. The functional images were then normalized to a standard EPI template using the same transformation and spatially smoothed with an isotropic 6 mm full width at half maximum (FWHM) Gaussian kernel.

Correction for physiological noise was performed via RETROICOR using Fourier expansions of different orders for the estimated phases of cardiac pulsation (3rd order), respiration (4th order) and cardio-respiratory interactions (1st order). The corresponding confound regressors, in addition to head movement confound regressors, were created using the Matlab PhysIO Toolbox (Kasper et al., 2017). If one out of four runs included head motion exceeding 2 mm, the run was removed and only three runs were used for the subject level analysis. If two or more runs included

head motion exceeding 2 mm the participant was excluded. So, all the participants or runs that we excluded from the imaging analysis had multiple instances of volume-by-volume displacement greater than 2 mm. In total eight participants (6 with overweight/obesity and 2 with healthy weight) were excluded and twelve participants had only three out of four runs included in the first level analysis. Moreover, two lean women were excluded because their images were not available due to a data storage issue. A total of 56 participants (25 before the scanner update and 31 after it) was used for the neuroimaging analysis (30 women with healthy weight and 26 with overweight-obesity).

fMRI data analysis - subject level

At the subject level the fMRI data were analysed using a mass-univariate approach based on General Linear Models (GLMs) in SPM12 (Kiebel, 2003; Poline, 2003). The BOLD time series in each voxel was used as the dependent variable. We concatenated the individual runs in one single run. We added regressors to account for the mean level of BOLD activity within each separate run and three more regressors capturing the beginning of each run (starting from the second run) to model the time point when a run stopped and the following one begun.

For each participant, we modeled the four main time periods within each trial: 1) visual cue presentation, 2) milkshake delivery, 3) rating scale onset, and 4) swallow instruction. The swallow instruction onset together with the visual cue were considered as events with a duration equal to zero. The milkshake delivery and rating scale onsets were modeled as boxcar functions with duration 2 sec and 3.5 sec, respectively. The shared variance (i.e. R-squared) between these two onset regressors was 0.28. Moreover, we added two parametric modulators. The first was at the onset of the rating scale and was equal to the reported rating value on that trial. The second

was at the time of milkshake delivery and was equal to 0 for low caloric milkshakes and 1 for high caloric milkshakes.

The 4 onset regressors plus the 2 parametric modulators were specified for each rating type (intensity, pleasantness and identification). Given that participants identified the milkshake correctly most of the time, we excluded the parametric modulator for the ratings in the identification task to avoid collinearity with the constant intercept. Thus, we had a total of 17 (2×6+5) regressors across the three rating types. The responses to the water trials were modeled separately, adding 5 more regressors (1 for delivery, 3 for ratings, 1 for swallowing). In total, the design matrix for each participant was comprised of 50 regressors, 22 onset regressors or parametric modulators, 24 movement or physiological regressors, 1 constant and 3 regressors to signal the beginning of runs 2, 3 and 4 (see Fig.A4 in the Appendix). Before including the onsets and parametric regressors in the GLM, we convolved them with the canonical hemodynamic response function (HRF) and sequentially orthogonalized them.

Using this GLM, we focused on BOLD responses at the time of milkshake delivery as a function of the milkshakes caloric content. To increase statistical power for the milkshake delivery contrasts, we collapsed across the question types (48 trials) shown in each separate satiety state and day. Our primary effects of interest were difference between high and low caloric milkshakes as a function of satiety state and BMI. In order to better understand potential interaction effects between Milkshake Type, Satiety State, and BMI, we also examined the two separate contrasts for high or low caloric milkshakes versus the fixation baseline.

fMRI data analysis - group level

At the group level we analyzed the imaging data with FSL v6.0 (Woolrich et al., 2009; Jenkinson et al., 2012), using the randomise tool (Anderson & Robinson, 2001)

to run permutation tests. The GLM for the group level analysis is given in Equation 2. This regression included dummy variables for 1) Satiety (fasted = 0, fed = 1) , 2) Day (day 1 = 0, day 2 = 1), and 3) cycle phase (preovulatory = 0, postovulatory = 1). It also included the Z-scored BMI as continuous covariate. This regression model included repeated measurements (4 sessions) for each participant. Therefore, we defined the exchangeability blocks so that the observations were permuted only within block, i.e. within the same participant.

$$BOLD_i = \beta_0 + \beta_1 Satiety_i + \beta_2 BMI_i + \beta_3 Satiety_i BMI_i + \beta_4 Day_i + \beta_5 CP_i \quad (2)$$

Permutation tests allowed us to make non parametric inferences at the voxel level after applying the Threshold-Free Cluster Enhancement (TFCE) algorithm (Smith & Nichols, 2009). We used the default values for TFCE applied to fMRI data and 5000 permutations as recommended to obtain a confidence limit for the nominal alpha of $p = 0.0500 \pm 0.0062$ (Winkler et al., 2014). To control for multiple comparisons across voxels either within an a priori defined set of hedonic brain regions or the whole brain, we applied family wise error correction ($p < 0.05$) at the voxel level after applying TFCE. We constructed a binary mask composed of canonical hedonic regions using the AAL atlas (Tzourio-Mazoyer et al., 2002), because of our a priori interest in this system. The included regions are consistently activated when people see pictures of food or taste food (Kringelbach & Stein, 2010; Carnell et al., 2012) and included orbitofrontal cortex (OFC), ventromedial prefrontal cortex (vmPFC), amygdala, putamen and caudate (i.e. dorsal striatum), nucleus accumbens (ventral striatum), thalamus, insular and olfactory cortex. All these regions are part of the hedonic system and encode the hedonic value of food. We corrected for multiple comparisons across voxels within this mask in our primary analyses. The binary mask is available together with the group-level contrasts at

p = 0.05 (https://identifiers.org/neurovault.collection:6871). For completeness, we also tested and report regions surviving corrections for multiple comparisons when considering all voxels throughout the brain. To label the brain regions we used the automatic labels from the Harvard Oxford atlas and visual inspection.

Results

Satiety manipulation

First, we ensured that the satiety manipulation induced by the overnight fast and ad libitum meal worked as expected. Indeed, we found that the ratings for satiety, hunger, and desire to eat differed in the fasted and fed states as expected (see Fig. 2).

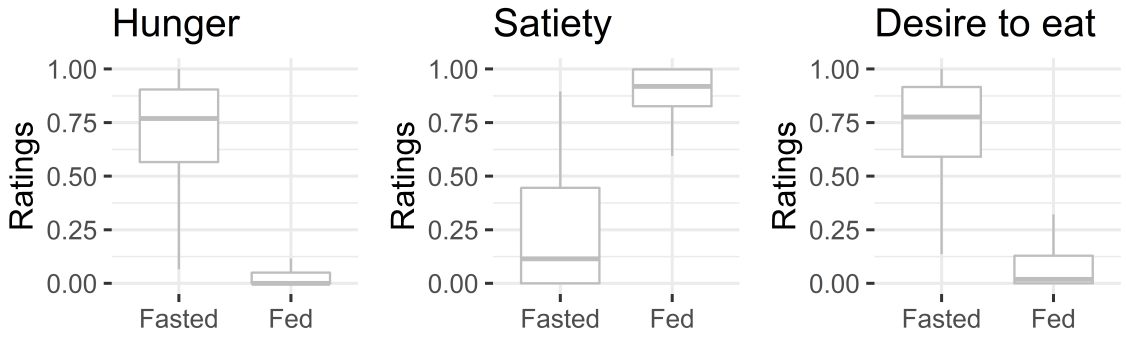

Figure 2: Verification of satiety manipulation. Standard boxplots show that satiety ratings increased after the ad libitum meal, while the ratings for hunger and the desire to eat decreased. In these boxplots, the thicker central mark indicates the median and the bottom and top edges of the box indicate the 25th and 75th percentiles, respectively. The whiskers extend to the most extreme data points not considered outliers (i.e. those within $\pm$ 2.7 standard deviations of the mean).

Experienced pleasantness ratings

To study hedonic responses to experienced food, we tested women with obesity and healthy weight while they consumed high and low caloric milkshakes in fasted and fed states. We used a generalized linear mixed-effects regression model to investigate how satiety, milkshake caloric content, and BMI related to the experienced

pleasantness of milkshakes, while controlling for several other potentially relevant factors (see Table 2). There was a significant interaction effect indicating that the differences in the pleasantness ratings in the fasted versus fed states depended on the calories in the milkshakes (mean coefficient of interaction between fed/fasted and milkshake type: -0.10, CI: [-0.19, -0.01], p = 0.025). When examining the nature of this interaction, we saw that the participants experienced low caloric milkshakes as less pleasant in the fed state versus fasted state, while ratings for high caloric milkshakes tended to increase when fed. However, the 3-way interaction between milkshake calories, fed/fasted state, and BMI was not significant.

Instead, the relationship between BMI and pleasantness ratings was conditional on a measure we refer to as the ad libitum consumption level. The ad libitum consumption level for each participant was defined as the grams/calories of food she consumed during the ad libitum lunch provided in the lab. We found that the relationship between hedonic responses and BMI differed as a function of ad libitum consumption level (interaction mean coefficient: 0.19, CI: [0.06, 0.33], p = 0.004, Table 2).

Specifically, in women with lower ad libitum consumption levels, pleasantness ratings decreased as a function of BMI. In contrast, in women with higher ad libitum consumption levels, pleasantness ratings increased as a function of BMI. Note that the interaction between BMI and ad libitum consumption levels did not significantly differ between the fasted and fed conditions. In fact, model comparisons showed that a regression model without the interaction term between fed/fasted state and ad libitum consumption level fit the data just as well as a version including the interaction (AIC difference = 0.86). Additional model comparisons showed that a model that included only fed/fasted (without ad libitum consumption level) fit our data less well (AIC difference = 13.06). These results indicate that the ad

libitum consumption level indexes something about the individual and/or state that is distinct from simply being in a fasted or fed state.

One possibility is that the relationship between experienced pleasantness, BMI, and ad libitum consumption level may be driven by different perceived hunger levels before and/or after the meal in women with healthy weight relative to women with overweight/obesity. We ran linear mixed effects regressions to test for relationships between perceived hunger levels, BMI, and the amount of calories eaten during the meal. The first model used BMI, perceived hunger levels before and after eating, as well as controls for day 1 versus day 2 and menstrual cycle phase to explain the amount of calories consumed during the ad libitum meal to reach satiety (Table 4). As expected, women with a higher BMI or higher perceived hunger levels consumed more calories (food) during lunch. Women with higher BMI showed an increase in calories to reach satiety (mean: 0.28, CI[0.06, 0.50], p = 0.018). Critically, perceived hunger levels after eating were not associated with the amount of food consumed. Thus, it is unlikely that ad libitum consumption level is simply a proxy for perceived hunger levels because it is not consistently related to hunger in either the pre and post-meal sessions. Recall that the 3-way interaction, MilkshakeLow*AdLibConsumLevel*BMI, does not differ between the fasted and fed test sessions.

We also ran a second set of three linear mixed effects regression model with perceived hunger levels before, after, or the change in hunger as the dependent variables. The purpose of these models was to test if women with obesity and high ad libitum consumption levels might have eaten more, but still felt hungry and, therefore, continued to value the milkshakes relatively more than women with healthy weight after eating. There was a main effect of ad libitum consumption level on the decrease in perceived hunger levels after the meal (mean: -0.10, CI[-0.15, -0.05], p = 0.0004).

	Pleasantness BMI-model
Fed	0.09 [-0.02,0.21]
MilkshakeLow	0.08 [-0.01,0.16]
TrialNumber	-0.04 * [-0.07,-0.01]
AdLibConsumLevel (Zscore)	-0.09 [-0.27,0.08]
BMI (Zscore)	-0.03 [-0.26,0.20]
Day2	0.15 * [0.01,0.28]
AfterOvulation	-0.10 [-0.21,0.01]
Prop	0.02 [-0.21,0.24]
Fed*MilkshakeLow	-0.10 * [-0.19,-0.01]
Fed*AdLibConsumLevel	0.06 [-0.03,0.14]
Fed*BMI	0.06 [-0.04,0.16]
Fed*Day2	-0.07 [-0.17,0.02]
MilkshakeLow*BMI	-0.02 [-0.11,0.07]
MilkshakeLow*AdLibConsumLevel	-0.09 ** [-0.16,-0.02]
AdLibConsumLevel*BMI	0.19 ** [0.06,0.33]
TrialNumber*MilkshakeLow	-0.01 [-0.06,0.03]
TrialNumber*BMI	-0.01 [-0.05,0.02]
AdLibConsumLevel*Day2	-0.05 [-0.18,0.07]
TrialNumber*BMI*MilkshakeLow	0.02 [-0.02,0.07]
MilkshakeLow*Fed*BMI	0.01 [-0.08,0.10]
MilkshakeLow*AdLibConsumLevel*BMI	-0.05 [-0.11,0.01]
Fed*AdLibConsumLevel*BMI	-0.07 [-0.14,0.00]
nobs	7435
sigma	3.19
logLik	2761.58
AIC	-5455.16
BIC	-5220.09
df.residual	7401.00

*** p < 0.001; ** p < 0.01; * p < 0.05.

Table 2: Milkshake pleasantness ratings as function of state (fasted, fed), milkshake type (high vs low calorie), BMI, and ad libitum consumption level. The table shows the mean estimates and the 95% confidence intervals for the coefficients from equation 1 (see Methods section) seeking to explain milkshake pleasantness ratings. The association between the experienced pleasantness for low caloric milkshakes and calories consumed during the ad libitum meal differed as a function of BMI (see Figure 3 for the effects driving this interaction). Other significant effects were independent of BMI and included a Fed * MilkshakeLow interaction, indicating that the difference in experienced pleasantness between low vs high caloric milkshakes disappeared or even partially reversed in the fed sessions. Moreover, participants who experienced low caloric milkshakes as relatively less pleasant ate more during the ad libitum meal (shown by the MilkshakeLow * AdLibConsumLevel interaction). The p-values were obtained using the Wald-Z statistic. Pleasantness = dependent variable. BMI range [18.8, 37.4], mean: 26.9, SD: 5.5. Ad Libitum Consumption Level range [136, 1072], mean: 568, SD: 209.

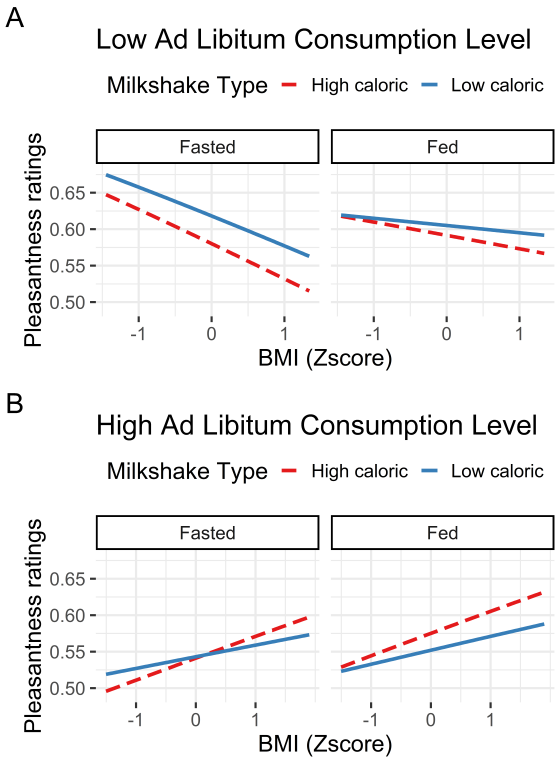

Figure 3: The association between the experienced pleasantness of milkshake rewards and amount eaten during an ad libitum meal differed between women with obesity and with healthy weight. These plots illustrate the AdLibConsumLevel * BMI interaction from the mixed-effects regression model reported in Table 2. (*A*) For low ad libitum consumption level (i.e. less food was needed to reach satiety), women with overweight/obesity tended to experience less pleasantness compared to women with healthy weight from both high and low caloric milkshakes (red dashed and blue solid lines, respectively). (*B*) In contrast, women with higher ad libitum consumption levels and higher BMI tended to experience the milkshakes as more pleasant, especially the high caloric ones. Here, we divided participants into low and high ad libitum consumption levels, relative to the group mean, for display purposes only. The regression analysis treated ad libitum consumption level as a continuous variable. The y-axis represents the group average pleasantness ratings as a function of BMI (x-axis) inferred from fits of the mixed-effect regression model. Note that there was no significant effect of being fed versus fasted on the relationship between ad libitum consumption level and BMI, indicating that ad libitum consumption likely indexes something beyond perceived hunger levels or a neural response to the ingested food.

	Change in Hunger Level	Hunger Level Before	Hunger Level After
AdLibConsumLevel (Zscore)	-0.10 *** [-0.15,-0.05]	0.09 *** [0.04,0.14]	-0.01 [-0.03,0.01]
BMI (Zscore)	0.03 [-0.03,0.08]	-0.04 [-0.09,0.01]	-0.01 [-0.03,0.00]
BMI* AdLibConsumLevel	-0.00 [-0.05,0.04]	0.01 [-0.03,0.05]	0.01 [-0.01,0.03]
Day 2	0.04 [-0.02,0.10]	-0.01 [-0.07,0.04]	0.02 [-0.00,0.05]
PostOvulatory	-0.03 [-0.09,0.03]	0.03 [-0.03,0.08]	-0.00 [-0.03,0.02]
N	130	130	130
N (participant)	66	66	66
AIC	23.19	3.36	-223.73
BIC	46.13	26.30	-200.79
R2 (fixed)	0.14	0.14	0.06

*** p < 0.001; ** p < 0.01; * p < 0.05.

Table 3: Control analysis of hunger levels. We report separately three models, the overall change in hunger level as well as the hunger level before and after eating. Both the change in hunger level and the hunger level before eating model show a dependency only on ad libitum consumption level. The higher the ad libitum consumption level, the stronger the change in hunger level (hunger decreased) and the higher the hunger ratings before eating. The hunger level after eating is independent of ad libitum consumption level, but increased on the second day. In all the three models there was no effect of BMI or interaction between BMI and ad libitum consumption level, showing that there were no hunger level differences between the women with overweight/obesity and the women with healthy weight.

However, there were no main effects or interactions with BMI on perceived hunger levels or their change (Table 3). Thus, these results argue against ad libitum consumption level being a simple proxy for hunger as related to its association with the experienced pleasantness of milkshakes in women with high BMI.

Overall, the experienced pleasantness participants reported for the milkshakes depended on the interplay the milkshake's caloric content, the participant's BMI, fasted/fed state, and her ad libitum consumption level.

	AdLibConsumLevel
BMI (Zscore)	0.29 ** [0.09,0.49]
Hunger Level Before	0.28 *** [0.13,0.42]
Hunger Level After	-0.06 [-0.18,0.07]
Day 2	0.35 *** [0.17,0.52]
PostOvulatory	0.09 [-0.09,0.27]
N	130
N (participant)	66
AIC	322.09
BIC	345.03
R2 (fixed)	0.20

*** p < 0.001; ** p < 0.01; * p < 0.05.

Table 4: Control analysis of differences in ad libitum consumption level. This table shows the results of a control regression testing how differences in ad libitum consumption level relate to BMI and hunger ratings. Ad libitum consumption levels were significantly related to BMI, perceived hunger levels before eating, and differed between the first and second days of the experiment. However, together these variables only explained approximately 20% of the individual variability in ad libitum consumption levels.

Neural responses to experienced pleasantness

At the neural level, we used fMRI to investigate brain activity as a function of BMI during high versus low caloric milkshake consumption before and after the meal. We performed these analyses both within a priori regions associated with eating and reward processing as well as throughout the whole brain. We focused our analysis on the time point when the milkshake was delivered and its pleasantness first experienced.

We tested our primary hypothesis that neural responses to high and low caloric food rewards depend on both satiety and BMI. Specifically, we tested for a 3-way interaction between BMI, Milkshake Type, and fasted vs fed states. We found significant 3-way interactions within both a priori regions of interest and other brain regions (Table 5). In particular, the responses of the nucleus accumbens, putamen, caudate, ventral medial prefrontal cortex, and thalamus differed between fasted and fed trials depending on BMI and Milkshake Type (Figure 4A). Note that in order to facilitate comparisons with other studies that do not include all variables necessary to compute the 3-way interaction, we also report correlations with BMI for each milkshake type (low and high caloric) and satiety condition separately in Table 6. To illustrate the data underlying the significant 3-way interaction effects, we extracted beta values from areas of the hedonic/reward system and plotted them in Figure 4B-E. The relationship between BMI and neural responses to milkshakes tended to be stronger (corresponding to steeper slopes) when fed compared to fasted. In other words, the neural responses in women with obesity differed more from those of women with healthy weight in fed trials than in fasted trials. These findings suggest that satiety affects neural responses in regions often associated with reward processing differently in women with obesity versus healthy weight.

Response to High vs. Low in Fasted vs. Fed as a function of BMI		Peak MNI Coordinates			
A PRIORI REGIONS	Peak Region Label	x	y	z	Peak t-value
	L Thalamus/Caudate	-12	-6	15	3.20
	L Putamen	-27	-9	0	2.48
	R Caudate	12	0	12	3.16
	R Thalamus	15	-30	12	2.87
	Nucleus Accumbens	-3	6	-6	3.00
	R Putamen	21	0	6	2.62
WHOLE BRAIN					
	R Medial Temporal Pole	51	15	-33	4.32
	Superior Temporal Gyrus, anterior division	63	0	-12	4.01
	Brain-Stem/Hypothalamus	3	-39	-18	3.97
	Frontal Pole	-33	60	-9	3.17
	Lateral OFC	-48	45	-15	2.88
	Inferior Frontal Gyrus, pars triangularis	-57	30	9	3.16
	Precuneus	15	-51	48	2.65
	Lateral Occipital Cortex, superior division	15	-69	51	2.57
	Posterior Cingulate Cortex	-6	-45	33	2.49
	R Superior Frontal Gyrus	21	60	24	2.49

Table 5: Neural responses to milkshakes reflecting interactions between BMI, Milkshake calories, and fasted versus fed state. The listed areas were more strongly activated by high caloric compared to low caloric milkshake before versus after eating in women with higher BMI. The table shows the peak coordinates of the clusters and the peak t-values.

In addition to the regions of the hedonic/reward system, other regions also showed similar 3-way interactions between BMI, Milkshake and being fed versus fasted. These included prefrontal cortex (IFG and superior frontal gyrus (SFG) and the hypothalamus (Figure 5A). Women with obesity once again a different activity pattern (Figures 5B-E) than healthy weight women in these regions. Interestingly, in women with obesity the hypothalamus (Figure 5B) showed weaker phasic responses to the milkshakes, both in fed and fasted states. This pattern of activity contrasts with that of the hedonic regions, where responses to milkshakes were stronger in women with obesity than with healthy weight (Figure 4). Together with the results above, these findings suggest potential dysfunctions in both hedonic and homeostatic systems in women with obesity.

Given our behavioural results on the influence of Ad libitum consumption levels on pleasantness ratings, we also tested for differences in the BOLD signal as a function of Ad libitum consumption levels. However, we did not find any significant associations.

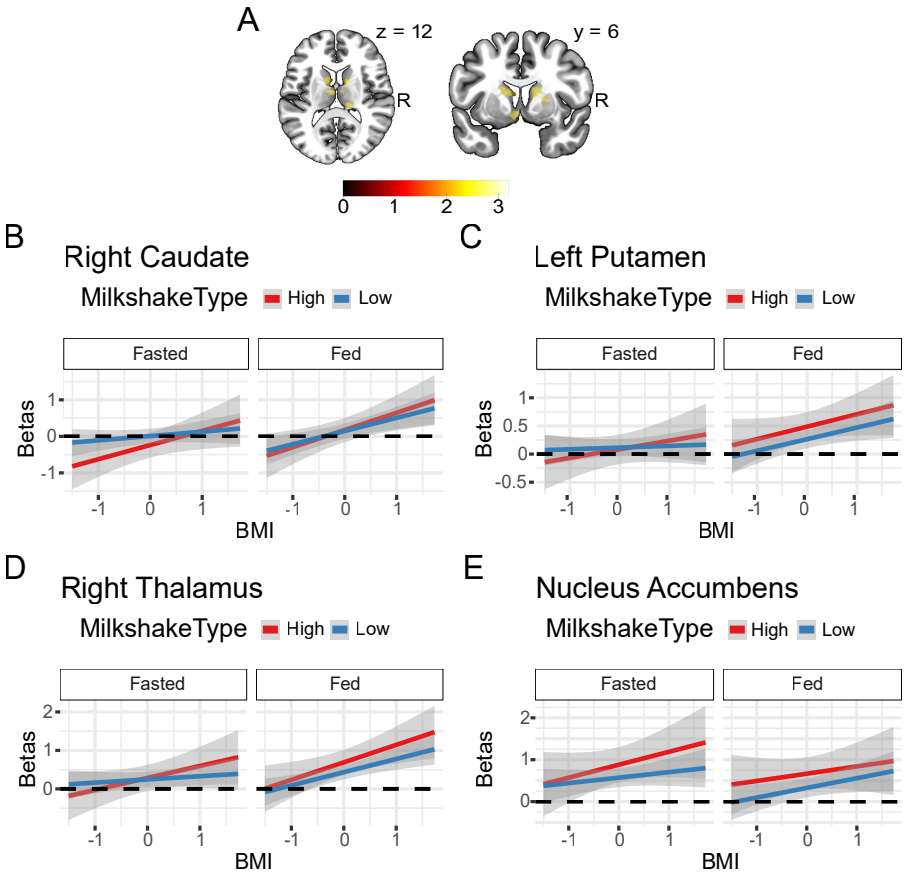

Figure 4: Neural responses to milkshakes reflecting interactions between BMI, Milkshake calories, and fasted versus fed state. A) Voxels where responses to high versus low caloric milkshakes depended on both satiety and BMI. The color scale indicates t-values derived from 5000 permutations of the data. The statistical parametric map is restricted to the a priori regions and small volume corrected ($p < 0.05$) after applying TFCE. B-E) Illustration of effects shown in Figure A for the caudate (B), putamen (C), thalamus (D), and nucleus accumbens (E). The responses to high (red) and low (blue) caloric milkshakes are plotted separately in the fasted and fed conditions as a function of BMI (z-scored). In healthy weight women, activity to the low caloric milkshake was stronger before the meal and showed greater decrease after eating to satiety. In contrast, women with obesity consistently showed stronger activity for high than low caloric milkshakes in both fasted and fed states, in line with reduced neural responses to satiety. Moreover, the activity differences between women with healthy weight and with obesity typically increased in the fed relative to fasted state (reflected by steeper slopes).

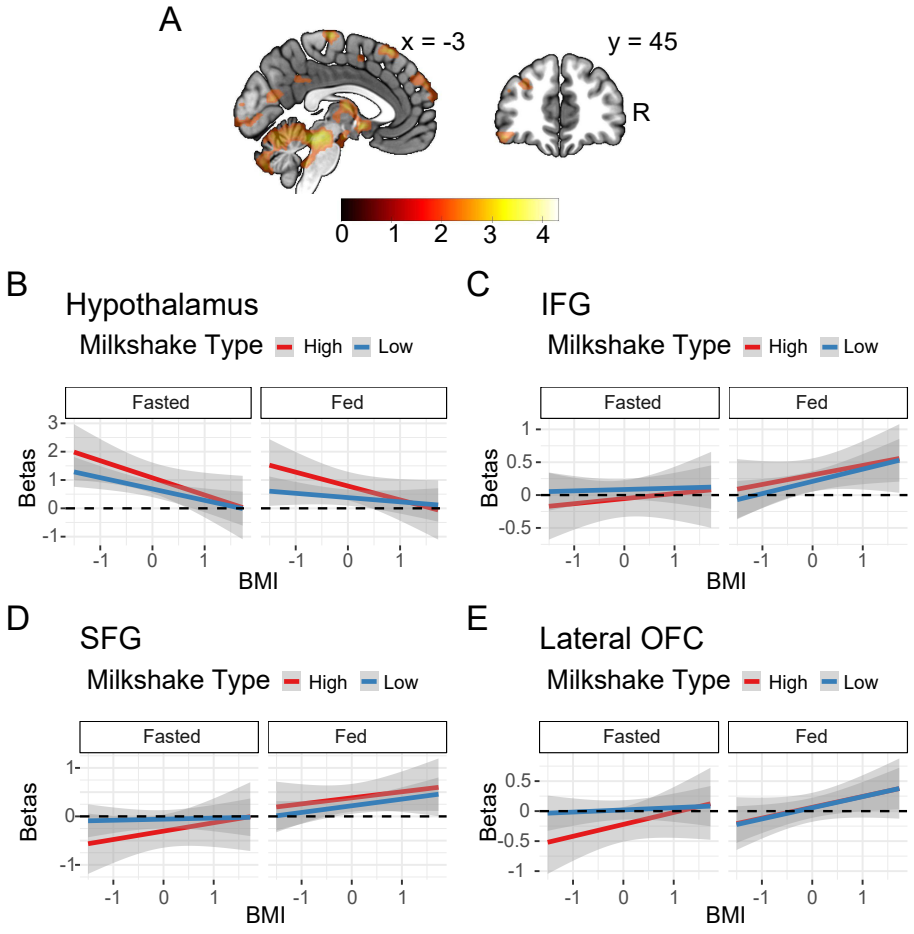

Figure 5: Further regions showing interactions between BMI, Milkshake calories, and fasted versus fed state. A) Voxels where responses to high versus low caloric milkshakes depended on both satiety and BMI. The color scale indicates t-values derived from 5000 permutations of the data. The statistical parametric map is whole brain corrected ($p < 0.05$) after applying TFCE. B-E) Illustration of effects shown in Figure A for the hypothalamus (B), IFG (C), SFG (D), and lateral OFC (E). The responses to high (red) and low (blue) caloric milkshakes are plotted separately in the fasted and fed conditions as a function of BMI (z-scored). Note that the hypothalamus responded more strongly to milkshakes and differentiated more between low and high caloric milkshakes in women with healthy weight compared to women with obesity. By contrast, all other regions show stronger responses to milkshakes in women with obesity than healthy weight, similar to the hedonic regions in Figure 4.

Response to High Caloric Milkshake as a function of BMI		Peak MNI Coordinates				Multiple contrasts correction
FASTED	Peak Region Label	x	y	z	Peak t-value	
	L Thalamus/Caudate	-9	-12	18	4.70	
	R Thalamus/Caudate	6	-12	15	4.19	
FED						
	R Cerebellum (VII)	42	-42	-45	5.20	*
	L Cerebellum (VIII)	-24	-39	-51	5.13	*
	L Thalamus	-15	-15	18	4.80	
	R Thalamus/Caudate	12	-12	18	4.17	
	R Insula	51	9	-9	3.80	
	R Rolandic Operculum	66	-24	21	3.53	
	R Superior Temporal Gyrus	48	-27	9	2.56	

Response to Low Caloric Milkshake as a function of BMI		Peak MNI Coordinates				
FASTED	Peak Region Label	x	y	z	Peak t-value	
	No significant effects					
FED						
	L Cerebellum (VIII)	-24	-39	-51	5.85	*
	R Cerebellum (VII)	42	-42	-45	5.60	*
	R Middle Temporal Gyrus/Insula	66	-12	-15	5.57	*
	L Thalamus/Caudate	-15	-15	18	5.22	
	L Angular Gyrus	-30	-57	42	4.27	
	Supramarginal Gyrus, anterior division	-42	-39	39	4.07	

Table 6: Effect of BMI on neural activity reported by fasted or fed status. These areas showed activity for high caloric and low caloric milkshakes that differed as a function of BMI in either the fasted or stated states. The peaks of the clusters are given in MNI space along with the peak t-values. Whole-brain corrected ($p < 0.05$) results are reported. No further voxels were found to be significant when applying small volume corrections within our a priori regions of interest. The * indicates which regions survived correction for multiple contrasts in addition to the correction for multiple voxels within the brain volume (original whole-brain corrected threshold divided by four, $p < 0.0125$).

Discussion

Our study directly tested the association between subjective pleasantness of experienced food rewards with different calorie contents and the amount of calories consumed during a separate meal as a function of BMI. We found that the pleasantness of milkshakes as well as the neural responses they trigger showed an interplay with the caloric composition, eating behavior, and weight status in women. Women with both higher BMI and ad libitum consumption levels reported greater experienced pleasantness for food rewards. Moreover, the hedonic system in women with higher BMI consistently showed stronger activity for high than low caloric milkshakes in both fasted and fed states, in line with reduced neural responses to satiety in women with obesity. These data shed new light on the behavioral and neural differences between women with healthy weight and women with obesity.

In our study, women with obesity who reported experiencing the milkshakes as more rewarding also ate more during the meal. Links between experienced pleasantness (i.e. reward) and calories consumed to reach satiety are postulated by both the reward deficit (Blum et al., 2014) and surfeit hypotheses of obesity (Davis et al., 2004; Stice et al., 2008), which are based on studies of the dopaminergic system (Stice et al., 2015; Kroemer & Small, 2016; Devoto et al., 2018). The surfeit hypothesis suggests that individuals at risk for obesity initially are more responsive to high caloric food. By contrast, according to the deficit theory the reward system is less responsive (Stice & Burger, 2019). The behavioral findings together with the consistently stronger responses of the hedonic system to high caloric foods appear more consistent with the reward surfeit hypothesis. However, our data also suggest that the reward system is less responsive to satiety in women with obesity than in women with healthy weight. In this regard, it is important to keep in mind that

there are multiple causes of obesity. Thus, even though reward deficit and surfeit are opposing hypotheses, it need not be the case that in people with obesity the brain is exclusively more or less responsive to food rewards. What is clear is that obesity is associated with altered food reward processing. Determining if, when, and how hypo- or hypersensitivity to rewards and internal states may increase the propensity for or result from obesity is an important ongoing aim.

The brains of women with obesity in our sample responded more strongly to high caloric milkshakes after the meal. These findings converge with those of Mason et al. (2019) who recently showed that individuals with obesity but not healthy weight attend more strongly to food cues after glucose consumption. They concluded that this attention bias could potentially lead individuals with obesity to continue consuming sugary foods because of difficulties in shifting attention away from food cues. However, it remains to be seen whether the attention bias also relates to amount of food consumed.

Surprisingly few neuroimaging studies have included direct comparisons of visual food cue reactivity or gustatory experiences in individuals with obesity relative to healthy weight during both the fasted and fed states. There have been two recent meta-analyses (Kennedy & Dimitropoulos, 2014; Devoto et al., 2018) of different food cue and consumption studies conducted using healthy weight and/or individuals with obesity. Both meta-analyses aggregated results over separate studies in order to test for reliable fasted and fed brain activity patterns, as well as potential BMI-dependent differences between the two conditions. However, there were only two data sets in Kennedy & Dimitropoulos (2014) and three in Devoto et al. (2018) that contained measurements of brain activity in both the fasted and fed states for both participants with healthy weight and participants with obesity. All of these tasks used visual images of foods rather than food consumption. A number of meta-analytically

significant differences were reported between participants with healthy weight and participants with obesity within either the fasted or fed conditions. However, both meta-analyses were more limited in their ability to detect reliable group (healthy weight, obese) by satiety interactions, perhaps because of the sparsity of within-subjects data. Thus, our study contributes important within-subjects data on the response to experienced food rewards in the fasted and fed conditions as a function of body mass. The importance of within-subjects data to investigate satiety effects has been highlighted in the past by a data set from Small and colleagues. In a series of papers on these data (Sun et al., 2014, 2015, 2016; Kroemer et al., 2016) the authors show how this type of data helps reveal biological (neuronal, hormonal, genetic) and psychometric links between responses to food reward and future gain of weight in individuals with obesity.

Our neural findings are more in line with previous reports and theories proposing that overeating might be triggered by higher, rather than lower, sensitivity to foods and food cues, especially for high caloric foods (Davis et al., 2004; Stoeckel et al., 2008; Stice et al., 2008). Our experienced food reward results are similar to the meta-analytic results from Kennedy & Dimitropoulos (2014) for food images in the fed condition, with higher activation in caudate and superior temporal gyrus in women with obesity compared to healthy weight when they experienced high caloric milkshakes or caudate and thalamus for the low caloric milkshakes. On the other hand, we did not find significant BMI-related differences in amygdala or hippocampus activity like they found for food images in the fasted condition. Instead, we again found higher caudate activation in women with obesity compared to healthy weight for the high caloric milkshakes during the fasted trials, similar to our findings in fed trials. These deviations from the aggregate pattern in Kennedy & Dimitropoulos (2014) might be due to the different nature of the experimental tasks (i.e. food cues

vs consumption).

In their meta-analysis, Devoto et al. (2018) report 2-way, group by modality (visual cue, gustatory consumption), as well as 3-way, group by modality by satiety, interactions in brain activity patterns. Our results are inconsistent with the apparent 3-way interactions in striatal BOLD activity reported in that meta-analysis. We observed sustained or increased activity in many sub-regions of the striatum in response to food rewards during the fed relative to the fasted condition. This is in contrast to the apparent pattern reported in Devoto et al. (2018) when meta-analytically aggregating results across 22 separate studies. Yet, there may be a simple reason for the inconsistency between our results and this meta-analysis. The bar plots in Figure 4 of Devoto et al. (2018) seem to show that there is "a near-zero" probability of observing activity in the caudate, nucleus accumbens, or ventral striatum more generally when women with obesity consume/taste food rewards in a fed state. However, the set of 22 papers in their meta-analysis does not include a single study looking at gustatory responses in individuals with obesity when they are fed. Therefore, there was no possibility for this meta-analysis to find a consistent activation in any brain region for fed individuals with obesity. This fact both explains the apparent inconsistency between the two sets of results and highlights the important new data and insights our study provides.

In addition to differences in neural response in reward-related regions, we found contrasting patterns between healthy weight and women with obesity in brain areas involved in maintaining homeostasis, such as the hypothalamus (Kullmann et al., 2014; Timper & Brüning, 2017). Within the hypothalamus, the BOLD responses decreased as a function of BMI for both high and low caloric milkshakes and across both fasted and fed trials. Thus, the hypothalamus showed less phasic (i.e. trial-specific) responses in fed women with obesity relative to women with healthy weight.

While the exact functional consequences of these phasic response patterns will require further investigation in future work, it is clear that the women with obesity in our sample had altered hypothalamic responses to individual food rewards and the state of satiety compared to the healthy weight women. These results suggest that individuals with obesity differ from healthy weight in terms of both hedonic and homeostatic responses to food when fed.

Women with obesity reported high satiety levels as well as low desire to eat and low hunger level after the ad libitum meal, but continued to show significant striatal and prefrontal cortex BOLD responses to food rewards in fed trials. In contrast, BOLD responses in these regions decreased during the fed condition in healthy weight women. This could mean that, 1) the brains of women with obesity respond differently to unaltered satiety signals from the periphery, or 2) that their brains receive reduced or altered signals. With regard to the second possibility, it may be that satiety information is not properly encoded at the gastrointestinal level in women with obesity. In this view, the hormones in the gut would have been less affected by the meal and, therefore, no or reduced signals passed on to the brain. In the absence of these signals, brain activity in response to the milkshakes would not differ between fed and fasted trials. This idea is consistent with studies on the hormone GLP-1, which is a potent incretin and may be involved in the control of appetite (Steinert et al., 2017). Meal-stimulated GLP-1 secretion was increased in individuals with obesity compared with individuals with healthy weight in several, but not all, studies (Steinert et al., 2017). Thus, either changes in peripheral secretion of GLP-1 or changes in the processing of GLP-1 signaling in the brain (ten Kulve et al., 2016) might lead individuals with obesity to perceive milkshakes (and food in general) as more valuable than individuals with healthy weight.

The role of potential menstrual cycle phase

Our results indicate that further investigation into the potential role of menstrual cycle phase in the experienced pleasantness of food rewards is warranted. In the primary specification of the model, using BMI we could not detect any statistically significant effect of cycle phase, but there was a trend ($p = 0.07$). Thus, we cannot exclude the possibility that cycle phase affects the pleasantness ratings. Indeed, when using WHR instead of BMI in a control analysis (see appendix Table A1), we found a significant effect of cycle phase, highlighting the importance of investigating menstrual cycle phase effects in future studies.

Strengths and limitations

Our work has several strengths and limitations. One strength is that our study included an ad libitum meal that measured the calories consumed to reach satiety. Data on actual eating behavior are key to understanding obesity in relation to both subjective experiences of reward and neural activity in response to food. A second strength is that we measured behavioral and brain responses to experienced food rewards in both the fasted and fed states for all participants. On the other hand, there are four important limitations of our work. First, we tested the subjective experiences and brain activity in response to a single type of food, milkshakes. Second, there is a timing confound within a single day because the fasted state is always followed by the fed state. Third, we examined only women. Fourth, we tested only individuals whose weight and body composition were fairly stable. Therefore, we cannot determine whether the differences we see are a cause or consequence of higher BMI. More research is needed to extend our findings and conclusions to other types of food and to males, and to determine the direction of causality.

Conclusion

Women with overweight or obesity compared to healthy weight showed stronger neural responses to food rewards even when fed. Similar or even increased neural responses to food rewards in fed compared to hungry states may be an important component of food overconsumption that leads to or perpetuates obesity.

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Authors Contributions

SG collected data, analyzed the data and wrote the paper, SCW programmed the task and collected data, GG and BL realized recruitment and selected the participants, DH collected the data. TAH and PNT provided guidance for the data analysis and contributed to the first draft of the paper. BL, LA, NG, PNT, SCW, and TAH designed the study. All authors contributed to the manuscript and approved the final version.

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Appendix

Participants

Here below we listed all the selection criteria

Inclusion:

- Physically and psychiatrically healthy women
- Lean women: BMI 18-25, weight in kg / height in m^2
- Obese women: BMI 30-35, weight in kg / height in m^2
- Stable body weight ($<$ 5 kg change in past year)
- Age 18-40 years
- Cycle history
- Right-handed
- German language fluency
- Signed informed consent

Exclusion:

- Life history of eating disorders
- Aversion to the test foods
- Pacemaker or neurostimulator
- Hearing aid
- Surgery to head or heart

- Potential metal parts in body (pacemakers, metal splinters, gun wounds, shrapnel or surgical clips)
- Neurological or psychiatric problems or serious brain injury (such as alcohol or drug abuse, depression, schizophrenia, bipolar disorders, anxiety disorder, claustrophobia, Parkinsons disease, multiple sclerosis, epilepsy)
- High blood pressure, low blood pressure, history of heart disease, irregular heart rate
- Emphysema, chest or respiratory problems (including difficulty breathing through the nose)
- Pregnancy, nursing or pregnancy planned in next three months
- History of gall bladder disease or symptoms (right upper abdominal quadrant pain after meals)
- Polycystic ovary syndrome, as determined by cycle history, clinical evaluation, transvaginally measured antral follicle count and hormonal measurements including testosterone
- Allergy or sensitivity to lactose
- Allergy to quinine
- Current or previous malignancies
- History of difficult blood sampling

Commercial milkshakes ingredients

The milkshakes were produced by Emmi and bought at Coop supermarket. Here we list the ingredients.

CHOCOLATE MILKSHAKE: Partly skimmed, fully pasteurized milk, 4% chocolate powder (sugar, cocoa powder, cocoa mass, cocoa butter), dextrose (2g / 100g), maltodextrin (2g / 100g), soluble fiber (inulin), stabilizer E331, flavor, concentrate milk minerals, thickener carrageenan , vitamins E, B6, B2, B1, D. 100 g contains 86 kcal.

STRAWBERRY MILKSHAKE: Partly skimmed, fully pasteurized milk, 8% concentrated strawberry juice, sugar, dextrose (2g / 100g), maltodextrin (2g / 100g), soluble fiber (inulin), stabilizer E339, rye juice concentrate, flavorings, concentrate milk minerals, thickener carrageenan, vitamins E, B6 , B2, B1, D. 100 g contains 86 kcal.

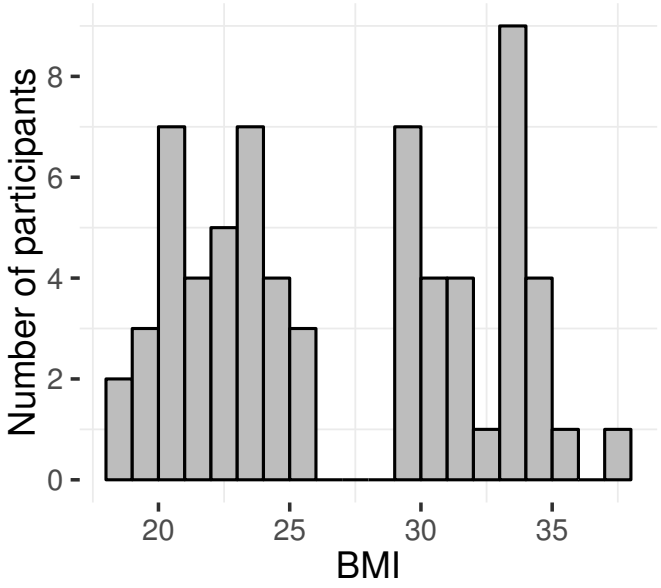

Figure A1: Distribution of the Body Mass Index. We report data from the 66 participants during the first scanner session (Day 1). Each bin comprises women with BMI ranging from x to < x+1. BMI of our sample: [18.8, 37.4], mean: 26.9, SD: 5.5.

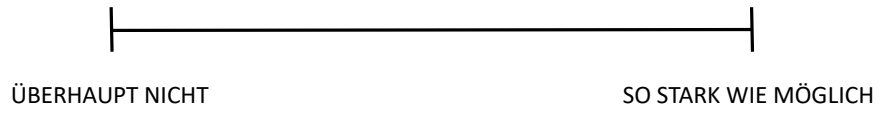

Figure A2: gVAS scale used for pleasantness ratings. The scale was continuous and anchored at the extremes, which were labeled respectively "not at all" and "as strong as possible".

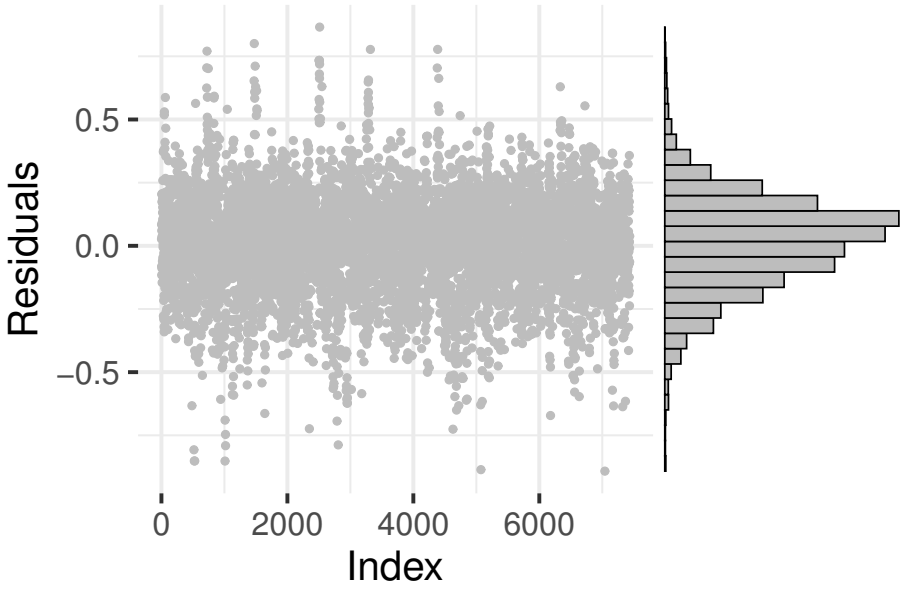

Figure A3: Visual inspection of the residuals from the glmmTMB model. The residuals show no obvious pattern and appear normally distributed.

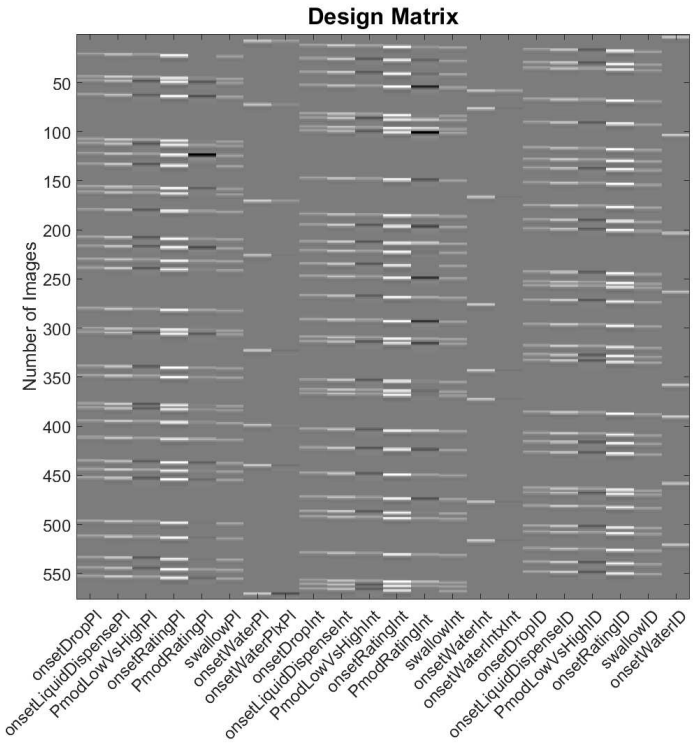

Figure A4: Example design matrix used at the fMRI subject level analysis. The 4 runs were concatenated for a total of 120 trials represented by the rows of the matrix. The columns contain the 22 regressors with onsets and parametric modulators. To make the matrix more readable we omit the 24 movement and physiological regressors, the 3 regressors to signal the beginning of run 2, 3 and 4, and the constant.

	Pleasantness BMI-model	Pleasantness WHR-model
Fed	0.09 [-0.02,0.21]	0.11 [-0.01,0.22]
MilkshakeLow	0.08 [-0.01,0.17]	0.09 * [0.01,0.17]
AdLibConsumLevel (Zscore)	-0.10 [-0.27,0.08]	-0.13 [-0.31,0.05]
BMI (Zscore)	-0.03 [-0.26,0.20]	
Day2	0.15 * [0.02,0.29]	0.16 * [0.03,0.30]
PostOvulatory	-0.10 [-0.21,0.01]	-0.11 * [-0.22,-0.00]
Prop	0.02 [-0.21,0.25]	0.03 [-0.20,0.25]
Fed*MilkshakeLow	-0.10 * [-0.19,-0.01]	-0.10 * [-0.19,-0.01]
Fed*AdLibConsumLevel	0.06 [-0.03,0.14]	0.09 [-0.00,0.17]
Fed*BMI	0.06 [-0.04,0.16]	
Fed*Day2	-0.07 [-0.17,0.02]	-0.08 [-0.17,0.02]
MilkshakeLow*BMI	-0.02 [-0.10,0.07]	
MilkshakeLow*AdLibConsumLevel	-0.09 ** [-0.16,-0.02]	-0.09 ** [-0.15,-0.02]
AdLibConsumLevel*BMI	0.19 ** [0.06,0.33]	
AdLibConsumLevel*Day2	-0.05 [-0.18,0.08]	-0.05 [-0.17,0.08]
MilkshakeLow*Fed*BMI	0.01 [-0.08,0.10]	
MilkshakeLow*AdLibConsumLevel*BMI	-0.05 [-0.11,0.01]	
Fed*AdLibConsumLevel*BMI	-0.07 [-0.14,0.00]	
WHR(Zscore)		-0.01 [-0.23,0.22]
Fed*WHR		-0.06 [-0.16,0.04]
AdLibConsumLevel*WHR		0.20 *** [0.08,0.32]
MilkshakeLow*Fed*WHR		0.05 [-0.04,0.14]
MilkshakeLow*AdLibConsumLevel*WHR		-0.10 ** [-0.15,-0.04]
Fed*AdLibConsumLevel*WHR		-0.10 ** [-0.17,-0.03]
nobs	7435	7435
sigma	3.18	3.18
logLik	2752.70	2760.46
AIC	-5445.40	-5460.92
BIC	-5237.98	-5253.50
df.residual	7405.00	7405.00

*** p < 0.001; ** p < 0.01; * p < 0.05.

Table A1: The table shows the results from generalized linear mixed effects models with respectively BMI and WHR as the measure of body composition. Here mean and 95 % confidence intervals are listed. The p-values were obtained using the Wald-Z statistic and we can see that the effects size of main findings are congruent between the two models. Pleasantness = dependent variable.